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Cambridge Studies in Mathematical Biology

Biological Kinetics

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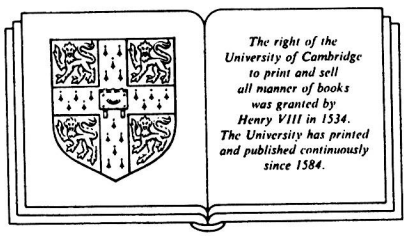
Biological kinetics



CAMBRIDGE UNIVERSITY PRESS
Cambridge
New York Port Chester Melbourne Sydney

CAMBRIDGE UNIVERSITY PRESS
Cambridge, New York, Melbourne, Madrid, Cape Town, Singapore, São Paulo

Cambridge University Press
The Edinburgh Building, Cambridge CB2 8RU, UK

Published in the United States of America by Cambridge University Press, New York

www.cambridge.org
Information on this title: www.cambridge.org/9780521391849

First published 1991
This digitally printed version 2008

A catalogue record for this publication is available from the British Library

Library of Congress Cataloguing in Publication data

Biological kinetics / edited by Lee A. Segel.
p. cm. — (Cambridge studies in mathematical biology : 12)
Includes index.
ISBN 0 521 39184 9
1. Molecular biology—Mathematical models. 2. Biophysics.
3. Molecular dynamics. I. Segel, Lee A. II. Series.
QH506.B543 1991
574.8'8'0151—dc20 90-2465 CIP

ISBN 978-0-521-39184-9 hardback
ISBN 978-0-521-06409-5 paperback

The central purpose of this book is to illustrate the premiss that theoretical analysis of the kinetics of biological processes can give valuable information concerning the underlying mechanisms that are responsible for these processes.

Topics covered range from cooperativity in protein binding and enzyme action, through receptor–effector coupling, to theories of biochemical oscillations in yeast and slime mold, of liver regeneration, and of neurotransmitter release. Theories are always closely coupled to experiment.

The material of this book originally appeared as part of the volume *Mathematical models in molecular and cellular biology* (edited by Lee A. Segel). However, each chapter has been revised and updated.

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PREFACE

The central purpose of this book is to illustrate the premiss that examination of the kinetics (time course) of biological processes can give valuable information concerning the underlying mechanisms that are responsible for these processes. To extract this information it is usually necessary to construct a mathematical model that embodies hypothesized mechanisms. Solution of the resulting equations shows whether the hypotheses are consistent with the data.

Considerable material concerns steady-state solutions. These can be regarded as the limiting behavior, in many instances, of the kinetic equations.

On the molecular level, the discourse ranges from fairly classical analyses of cooperativity in protein binding and enzyme action, through studies of enzyme induction and receptor–effector coupling, to theories for biochemical oscillations in yeast and slime mold. Models for the triggering of secretion in slime mold and in nerve cells, and for liver regeneration, are at the intersection of molecular biology, cellular biology and physiology. In addition, an introduction to the explosively growing theoretical topic of chaos concludes with references that chronicle tentative attempts to apply chaos theory in physiology (cardiac dynamics and immunology).

The material of this book originally appeared as part of *Mathematical models in molecular and cellular biology* (Lee A. Segel, ed., Cambridge: Cambridge University Press, 1980), which is now out of print. Each contribution has been revised and updated. (Unfortunately, Sol Rubinow has passed away. His contribution appears with permission of his widow, Shirley Rubinow, and has been updated by Lee A. Segel.)

The mathematical requisite for most of the material is a good command of basic calculus. A brief summary of the required mathematical ideas can be found in the Appendices of 'MDP', *Modelling dynamic phenomena in molecular and cellular biology* by Lee A. Segel (Cambridge: Cambridge University Press, 1984). Indeed, one of the uses to which the present book might be put is as a supplement to MDP, or to other texts in theoretical biology. There is substantial overlap with MDP in only one topic, cAMP

secretion in slime mold. However, the coverage of this topic in the present volume – although perhaps less detailed mathematically – is more comprehensive and up-to-date.

It is hoped that this volume will be of interest to students and researchers alike, in both biology and applied mathematics. Readers should find a number of interesting case studies that show how mathematical modeling can illuminate important areas of modern biology.

CONTENTS

1

Fundamental concepts in biochemical reaction theory

Law of mass action

Consider a reaction in which a chemical A of concentration A combines reversibly with a chemical B of concentration B to yield **complex**, C, of concentration C. This reaction is symbolized by

$$\mathrm{A} + \mathrm{B} \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} \mathrm{C}. \tag{1}$$

The **forward and backward rate constants** k_{+1} and k_{-1} are the proportionality factors in the **law of mass action** that is assumed to describe the process of the reaction. According to this law, the rate at which the species A reacts to form C is proportional to the mass of A, or equivalently, to the number of molecules of A available for reaction. In mathematical terms, the law takes the form of the following differential equations for the concentrations A, B, and C, at time t;

$$\mathrm{d}A/\mathrm{d}t = -k_{+1}AB + k_{-1}C, \qquad \mathrm{d}B/\mathrm{d}t = -k_{+1}AB + k_{-1}C, \tag{2a, b}$$

$$\mathrm{d}C/\mathrm{d}t = k_{+1}AB - k_{-1}C. \tag{2c}$$

In (2a), A is supposed to decrease at a rate jointly proportional to the concentrations of A and B. The idea behind this is again the law of mass action: doubling the concentrations of either A or B will double the rate of collision between these two molecules and hence will double the rate of 'successful' collisions that lead to the formation of C. Such an assumption is plausible as long as the concentrations are not too large. The break-up of an individual C molecule into its constituents is held to occur with a constant probability per unit time.

The phenomenological law of mass action can, in principle, be derived from statistical mechanics, or on a deeper level from quantum mechanics, but this law can be regarded as being well established because of experimental information on a wide variety of theories in the biological, chemical and physical sciences that assume it.

Enzyme–substrate complex system

Enzymes are large molecules that speed up the conversion of a chemical to an altered form. According to the theory of enzymatic reactions of Michaelis & Menten (1913), the enzyme accomplishes this in two steps. First the enzyme (concentration E) reacts reversibly with the chemical, called a **substrate** in this context, to form a **complex** (concentration C). Secondly, the complex breaks apart into an altered substrate or **product** and the original enzyme. This last reaction is often assumed to be irreversible, in which case one writes

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} C \xrightarrow{k_{+2}} E + P.$$

The law of mass action for the concentrations $E(t)$, $S(t)$, $C(t)$, and $S(t)$ takes the form

$$\mathrm{d}E/\mathrm{d}t = -k_{+1}ES + k_{-1}C + k_{+2}C, \tag{3a}$$

$$\mathrm{d}S/\mathrm{d}t = -k_{+1}ES + k_{-1}C, \tag{3b}$$

$$\mathrm{d}C/\mathrm{d}t = k_{+1}ES - k_{-1}C - k_{+2}C, \tag{3c}$$

$$\mathrm{d}P/\mathrm{d}t = k_{+2}C. \tag{3d}$$

The above **system of differential equations** representing the enzymatic conversion of substrates to product was first put forward by Briggs & Haldane (1925). The equation must be supplemented by **initial conditions** that describe the system at some reference time. This time is conveniently designated $t = 0$. The standard initial conditions, which conform to the usual investigation of enzymatically controlled reactions, prescribe starting concentrations of enzyme and substrate, and assume that complex and product have had no opportunity to form:

$$E(0) = E_0, \qquad S(0) = \mathrm{S}_0, \qquad \mathrm{C}(0) = 0, \qquad P(0) = 0. \tag{4}$$

Addition of (3*a*) and (3*c*) yields

$$\mathrm{d}(E + C)/\mathrm{d}t = 0. \tag{5}$$

Consequently $E + C$ must be a constant, reflecting the fact that at any time t all enzyme molecules are either in their original form or bound in a complex. Using the initial conditions, the constant can be determined, so that we can write the **conservation equation**

$$E(t) + C(t) = E_0. \tag{6a}$$

This equation may be used to eliminate E from (3*b*) and (3*c*), leaving two equations for the two unknown functions $S(t)$ and $C(t)$:

$$dS/dt = k_{+1}E_0S + C(k_{+1}S + k_{-1}), \tag{6b}$$

$$dC/dt = k_{+1}E_0S - k_{+1}C(S + K_m), \tag{6c}$$

$$K_m \equiv (k_{-1} + k_{+2})/k_{+1}. \tag{6d}$$

Pseudo-steady state: Michaelis–Menten equation

In laboratory experiments, it is typically the case that, at the start, many substrate molecules are present for each enzyme molecule. Under these circumstances one expects that after an initial short transient period there will be a balance between the formation of complex by the union of enzyme and substrate and the breaking apart of complex (either to enzyme and substrate, or to enzyme and product). Because there are so many substrate molecules, this balance will be achieved before there is perceptible transformation of substrate into product. One anticipates, therefore, that calculation of product formation can be carried out under the assumption that $dC/dt = 0$, or, from (3*c*),

$$k_{+1}ES = (k_{-1} + k_{+2})C. \tag{7}$$

This equation is said to result from a **quasi-** or **pseudo-steady state hypothesis**. If any quantity no longer changes with time it is said to be in a **steady state**. We add 'pseudo' or 'quasi' to the description of (7) as a steady state, since although C is fully adjusted to the instantaneous values of E and S, those values are changing slowly with time.

Upon substitution of (6*a*) and (7) into (3*b*), we obtain the following equation for S:

$$dS/dt = -k_2E_0S/(K_m + S). \tag{8}$$

The solution of (8) (by the method of separation of variables) subject to the initial condition $S(0) = S_0$, is

$$S + \frac{k_{-1} + k_{+2}}{k_{+1}} \ln \frac{S}{S_0} = S_0 - k_{+2}E_0t.$$

Of particular interest is the velocity of reaction $V(t)$ defined as the rate of appearance of product. In view of the steady state hypothesis, we have from (6*a*), (7) and (3*d*) that

$$V(t) = dP/dt = k_{+2}C = |dS/dt|. \tag{9}$$

Biochemists are usually interested in $V(t)$ at the beginning of the reaction. From (8) we can write for this **initial velocity** $V_0 \equiv V(0)$,

$$V_0 = \frac{VS_0}{K_m + S_0}, \tag{10a}$$

where

$$V \equiv k_{+2}E_0. \tag{10b}$$

Equation (10*a*) is called the **Michaelis–Menten** equation. Its graph starts from the origin, for the absence of substrate implies the absence of reaction, and approaches the asymptote $V_0 = V$ as S_0 becomes larger and larger (see Figure 1.1). Thus, when S_0 is large compared to K_m and $V_0 \approx V$, there is an abundance of substrate and the 'chemical factory' is working as fast as possible. In such cases the system is said to be **saturated**. Because the constant V is the maximum velocity that the reaction can attain, the term 'V-max' is used to describe it. (The term **Langmuir isotherm** is also associated with (10*a*), which is said to have the form of a **rectangular hyperbola**.)

The biochemical determination of the **Michaelis constant** K_m follows from the observation that when $S_0 = K_m$ then $V_0 = \frac{1}{2}V$. Thus K_m gives the concentration at which the reaction attains its half-maximal value. If this concentration is relatively low, then the reaction is said to be highly **specific**. A relatively low K_m means a relatively large k_{+1} and this in turn means that an enzyme–substrate collision is relatively likely to result in the formation of

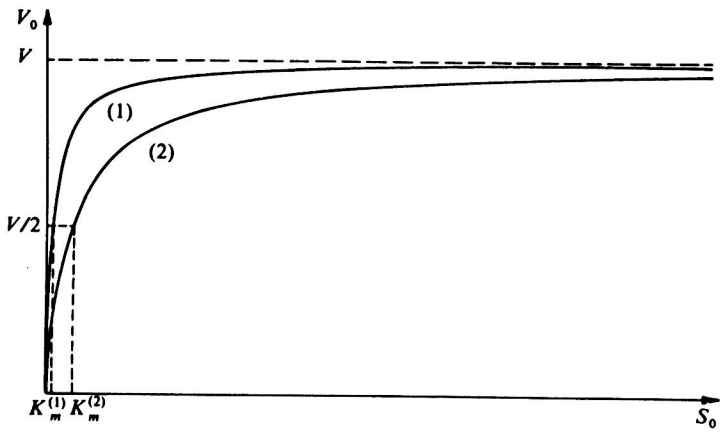

Figure 1.1. Graphs of the Michaelis–Menten equation (10*a*) in two situations with the same maximum velocity V. The reaction represented by curve (1) is more specific than that of curve (2) because the Michaelis constant for it is smaller:

$$K_m^{(1)} < K_m^{(2)}.$$

product, i.e. that the enzyme is specifically adapted to act on the particular substrate.

Biochemists frequently rearrange the Michaelis–Menten equation (10*a*) into the **Lineweaver–Burk** or **double-reciprocal** form

$$\frac{1}{V_0} = \frac{1}{V} + \left(\frac{K_m}{V}\right)\frac{1}{S_0}. \tag{11}$$

The graph $1/V_0$ versus $1/S_0$ is thus a straight line, which simplifies the problem of fitting the theory to data. Then $1/V$ and $-1/K_m$ can be found at once as the intersection of this line with the vertical and horizontal axes, respectively (Figure 1.2).

Note from (10*b*) that V depends on the product of the initial enzyme concentration E_0 and the product formation rate constant k_{+2}. This reflects the fact that at high substrate concentrations the speed of reaction depends only on how many reaction units there are, and on how fast they can transform complex into product. Under such circumstances one says that the enzyme is the **rate-limiting chemical** and the complex–product conversion is the **rate-limiting step** in the conversion of substrate to product.

The back reaction for the conversion of complex to product can also be included in the theory. Further, Haldane (1930) has indicated that the reaction of product and substrate should be viewed symmetrically so that the complete set of reactions presumed to take place is represented as

$$S + E \underset{k_{-1}}{\overset{k_{+1}}{\rightleftarrows}} C \underset{k'_{-2}}{\overset{k'_{+2}}{\rightleftarrows}} C' \underset{k_{-2}}{\overset{k_{+2}}{\rightleftarrows}} P + E, \tag{12}$$

where C is an S–E complex, and C' is a P–E complex. The analysis of this reaction scheme does not alter the form of the Michaelis–Menten equation (10*a*), although the meanings of V and K_m in terms of fundamental rate constants are more complicated than indicated by (10*b*) and (6*d*).

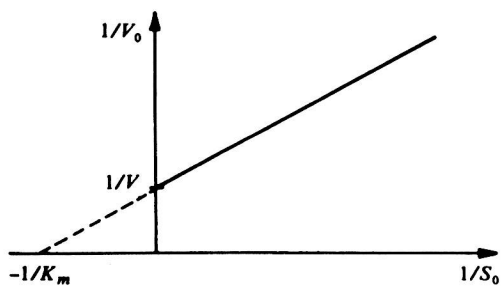

Figure 1.2. The Lineweaver–Burk plot, from which V and K_m can be readily determined. (The dashed part of the line corresponds to 'unphysical' negative substrate concentrations.)

The assumption of a pseudo-steady state can simplify a wide variety of kinetic problems. The most elementary application of this assumption yields the Michaelis–Menten equation (10*a*) that is a keystone of theoretical biochemistry. For both of these reasons it is worth carefully working out the conditions under which the pseudo-steady state assumption (7) is expected to be valid.

The key concept here is that of 'time scale', the order of magnitude of time that characterizes the duration of a process or subprocess. For example, what is the time scale of the fast transient process during which the complex concentration changes from its initial value of zero to a pseudo-state condition? Does it take microseconds, milliseconds or seconds? To *estimate* the duration of this period we can make the approximation $S = S_0$ in (6*c*). This transforms (6*c*) into a linear equation, with the solution

$$C(t) = \bar{C}[1 - \exp(-\mu t)], \qquad \mu \equiv k_{+1}(S_0 + K_m),$$
$$\bar{C} \equiv E_0 S_0/(K_m + S_0). \tag{13a, b, c}$$

Thus the *complex* (*fast*) *time scale* is given by $t_C = \mu^{-1}$:

$$t_C = [k_{+1}(S_0 + K_m)]^{-1}. \tag{14}$$

Now let us estimate the *substrate* (*slow*) *time scale* t_S, namely how long it takes for a significant change to occur in the substrate concentration. We employ the characterization (Segel 1984, p. 56).

$$t_S \approx \frac{\text{total change in } S \text{ after fast transient}}{\max |dS/dt| \text{ after fast transient}}. \tag{15}$$

The numerator of (15) is approximately S_0. Assuming the validity of the steady state assumption, we observe that the denominator is given by (8) with $S = S_0$. Thus $t_S \approx S_0/[k_{+2}E_0S_0/(K_m + S_0)]$, i.e.

$$t_S = (K_m + S_0)/k_{+2}E_0. \tag{16}$$

One necessary criterion for the validity of the pseudo-steady state assumption is that the 'fast transient' is indeed brief compared to the time during which the substrate changes appreciably. This criterion is $t_C \ll t_S$ or, from (14) and (16)

$$\frac{E_0}{K_m + S_0} \ll \left(1 + \frac{k_{-1}}{k_{+2}}\right)\left(1 + \frac{S_0}{K_m}\right). \tag{17}$$

A second criterion concerns the 'initial' condition $S(0) = S_0$ that is imposed on (8). For this condition to be approximately valid there must be only a negligible decrease in substrate concentration during the duration t_C

of the brief transient. This decrease, which we denote by ΔS, is certainly less than the product of the time duration t_C and the initial (maximal) rate of substrate consumption. 'Initial' in the previous sentence refers to the very beginning of the experiment, so that the desired rate is obtained by setting $t = 0$ in (6*b*). This yields

$$\left|\frac{\Delta S}{S_0}\right| = \frac{1}{S_0}\left|\frac{dS}{dt}\right|_{\max} \cdot t_C = \frac{E_0}{K_m + S_0}. \tag{18}$$

The requirement that $|\Delta S/S_0|$ be small compared to unity is thus expressed by

$$\varepsilon \ll 1, \qquad \text{where } \varepsilon \equiv \frac{E_0}{K_m + S_0}. \tag{19}$$

If (19) holds then (17) holds. Thus $\varepsilon \ll 1$ is a simple criterion for the validity of the pseudo-steady state assumption.

For considerable further discussion along the above lines see Segel (1988) and Segel & Slemrod (1989).

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